

This listing of the claims will replace all prior versions, and listings, of claims in the application:

LISTING OF THE CLAIMS

Claim 1 (currently amended): A method for immobilizing a biological molecule in a porous inorganic matrix, said method comprising:

forming an aqueous composition comprising a ceramic oxide colloidal sol mixed with an acidified oxide salt solution;

adding to said composition an amount of the biological molecule in a physiologically acceptable-buffered solution, said aqueous composition becoming turbid on being transformed into a polymerizing hydroxide solution and transforming to a gel;

shaping the gel produced in step (b) into a final form; ~~and~~

aging the gel; and

crushing the aged gel into particulates, wherein the crushed gel particulates are between about 10 μm and about 80 μm in diameter and are suitable for incorporation into a microanalytical device;

wherein said biological molecule is entrapped within pores of the gel, and the activity of the biological molecule is retained.

Claim 2 (original): The method of claim 1, wherein the gel is aged for about two weeks.

Claim 3 (original): The method of claim 1, wherein the gel is aged at a temperature of from about 4°C to about 40°C.

Claim 4 (original): The method of claim 1, wherein the pH of the mixture after the addition of the biological molecule is between about 6.0 and about 8.5.

Claim 5 (original): The method of claim 1, wherein the pH of the mixture after the addition of the biological molecule is between about 4.0 and about 7.0.

Claim 6 (original): The method of claim 1, wherein the pH of the mixture after the addition of the biological molecule is between about 7.0 and 9.0.

Claims 7-8 (canceled,

Claim 9 (currently amended): A method for immobilizing a biological molecule in a porous inorganic matrix incorporated into a microanalytical device, said method comprising:

forming an aqueous composition comprising a tetraalkyl orthosilicate and a silane, wherein the silane is substituted with a C₈-C₂₄ alkyl group and substituted with at least two leaving groups selected from OR and halo, ~~ceramic oxide colloidal sol and a dissolved metal silicate~~ mixed with an acidified oxide salt solution;

adding to said composition an amount of the biological material in a physiologically acceptable-buffered solution wherein the resulting aqueous composition has a pH ranging from about 6 to about 8.5, said aqueous composition becoming turbid on being transformed into a polymerizing hydroxide solution and transforming to a gel;

shaping the gel produced in step (b) into a final form; and

aging the gel;

wherein said biological molecule is entrapped within pores of the gel, and the activity of the biological molecule is retained; and wherein the porous inorganic matrix is formed in situ.

Claims 10-13 (canceled).

Claim 14 (currently amended): The method of claim ~~9~~ 13, wherein the alkyl group is C₁₈.

Claim 15 (currently amended): The method of claim ~~9~~ 12, wherein the tetraalkyl orthosilicate is selected from the group consisting of tetra-ethyl orthosilicate (~~TEOS~~), tetra-methyl orthosilicate (~~TMOS~~), and combinations thereof.

Claim 16 (original): The method of claim 1, wherein the sol is comprised of colloidal silica sol and a dissolved metal silicate.

Claim 17 (original): The method of claim 16, wherein the metal silicate is sodium silicate.

Claim 18 (original): The method of claim 1, wherein the sol comprises a tetraalkyl orthosilicate and a silane substituted with at least two leaving groups selected from the group consisting of OR and halo.

Claim 19 (original): The method of claim 18, wherein the silane is substituted with a C₈-C₂₄ alkyl group.

Claim 20 (original): The method of claim 19, wherein the alkyl group is C₁₈.

Claim 21 (currently amended): The method of claim 18, wherein the tetraalkyl orthosilicate is selected from the group consisting of tetra-ethyl orthosilicate (TEOS), tetra-methyl orthosilicate (TMOS), and combinations thereof.

Claims 22-23 (canceled).

Claim 24 (original): The method of claim 1, wherein the particle size of the ceramic oxide colloidal sol is selected to produce pores when the gel is aged, said pores being of a diameter which is approximately the same as the diameter of the biological molecule to be entrapped.

Claim 25 (canceled).

Claim 26 (original): The method of claim 24, wherein the pores have an average diameter ranging from about 1 nm to about 100 nm.

Claim 27 (original): The method of claim 1, wherein the pores have an average diameter ranging from about 2 nm to about 50 nm.

Claim 28 (currently amended): The method of claim 9, wherein the ~~particle size of the ceramic oxide colloidal sol is selected to produce pores when the gel is aged, said pores~~ have being of a diameter which is approximately the same as the diameter of the biological molecule to be entrapped.

Claim 29 (original): The method of claim 28, wherein the diameter of the pores is less than the diameter of the entrapped biomolecule.

Claim 30 (original): The method of claim 9, wherein the gel produced in step (b) is shaped into forms selected from the group consisting of a monolithic gel, thin film, or fiber.

Claim 31 (original): The method of claim 9, wherein the pores have an average diameter ranging from about 1 nm to about 100 nm.

Claim 32 (original): The method of claim 31, wherein the pores have an average diameter ranging from about 2 nm to about 50 nm.

Claim 33 (original): The method of claim 24, wherein molecules having a mass of 3,000 Da or less can diffuse through the pores.

Claim 34 (original): The method of claim 24, wherein molecules having a mass of 5,000 Da or less can diffuse through the pores.

Claim 35 (original): The method of claim 24, wherein molecules having a mass of 10,000 Da or less can diffuse through the pores.

Claim 36 (original): The method of claim 24, wherein molecules having a mass of 15,000 Da or less can diffuse through the pores.

Claim 37 (original): The method of claim 28, wherein molecules having a mass of 3,000 Da or less can diffuse through the pores.

Claim 38 (original): The method of claim 28, wherein molecules having a mass of 5,000 Da or less can diffuse through the pores.

Claim 39 (original): The method of claim 28, wherein molecules having a mass of 10,000 Da or less can diffuse through the pores.

Claim 40 (original): The method of claim 28, wherein molecules having a mass of 15,000 Da or less can diffuse through the pores.

Claim 41 (original): The method of claim 1, wherein the biological molecule is selected from the group consisting of polynucleotides, enzymes, antibodies, coagulation modulators, cytokines, endorphins, peptidyl hormones, kinins, receptors, genes, gene fragments, cell fragments, membrane fragments, and solubilized membrane proteins.

Claim 42 (currently amended): The method of claim 41, wherein the enzyme is selected from the group consisting of RNase, DNase, telomerase, ligase, nuclease, ribonuclease; hydrogenase, dehydrogenase, aldase, amidase, aminotransferase, amylase, anhydrase, apyrase, arginase, aspartase, aspariginase, carboxylase, carboxypeptidase, catalase, cellulase, cholinesterase, acetylcholinesterase, deaminase, dextranase, dismutase, elastase, esterase, fumarase, glucosidase, hexokinase, isomerase, invertase, kinase, lactase ~~laetasee~~, lipase, lysozyme, malase, naringinase, oxidase, oxygenase, papain, pectinase, peptidase, pepsin, peroxidase, phosphodiesterase, phosphotase, protease, reductase, transferase, tyrosinase, urase, trypsin, chymotrypsin, hydrolases, isomerases, proteases, ligases and oxidoreductases such as esterases, phosphatases, glycosidases and peptidases, superoxide dismutase (~~SOD~~), tissue plasminogen activator (~~TPA~~), renin, adenosine deaminase, alpha-glucocerebrosidase, asparaginase, dornase-alpha, hyaluronidase, elastase, trypsin, thymidine kinase (~~TK~~), tryptophan hydroxylase, urokinase, kallikrein, bromelain, cathepsins B, D, G, C, clostripain, endoproteinase Arg C, endoproteinase Asp N, endoproteinase Glu C, endoproteinase Lys C, Factor Xa, proteinase K, subtilisin, thermolysin, acyloamino acid releasing enzyme, aminopeptidases, carboxypeptidases, and pyroglutamate aminopeptidase.

Claim 43 (original): The method of claim 1, wherein the colloidal sol particle size is from about 1 nm to about 30 nm.

Claim 44 (original): A method of preparing a microanalytical device, comprising forming a sol-gel comprising an entrapped biological molecule, crushing the sol-gel to particulates having a diameter of from about 10 μm to about 80 μm , and forming the sol-gel particulates into a bed within the microanalytical device or on the surface of the microanalytical device.

Claim 45 (original): A method of preparing a microanalytical device comprising forming a sol-gel comprising an entrapped biological molecule, wherein the form of said sol-gel is selected from the group consisting of a monolithic gel, thin film, or fiber and wherein the sol-gel is placed in or on the microanalytical device.

Claim 46 (currently amended): A method of using a microanalytical device comprising a sol-gel comprising an entrapped biological molecule, comprising forming the sol-gel into a bed within the microanalytical device or on the surface of the microanalytical device, applying an analyte sample to the bed, optionally applying additional buffer solution to the bed, and analyzing the eluant from the bed.

Claim 47 (currently amended): The method of claim 44 or ~~46~~ 45, wherein the bed on the microanalytical device is in the form of a microcolumn or microchannel.

Claim 48 (currently amended): The method of claim 44 or ~~46~~ 45, wherein the bed on the microanalytical device is in the form of a microarray.

Claim 49 (original): The method of claim 46, wherein the eluant is analyzed using mass spectrometry.

Claim 50 (original): The method of claim 46, wherein the eluant is analyzed using micro or capillary electrophoresis.

Claim 51 (currently amended): The method of claim 46, wherein the interaction of any component in the sample with the entrapped biological molecule in the sol-gel is measured using a method selected from the group consisting of UV/Visible, Near IR, fluorescence, refractive index (~~RI~~) and Raman spectroscopies.

Claim 52 (original): The method of claim 46, further comprising washing the sol-gel with a solution to elute analytes from the sol-gel, and analyzing the analytes.

Claim 53 (original): The method of claim 52, wherein the analytes are analyzed using mass spectrometry.

Claim 54 (currently amended): The method of claim 52, wherein the analytes are analyzed using a method selected from the group consisting of UV/Visible, Near IR, fluorescence, refractive index (~~RI~~) and Raman spectroscopies.

Claim 55 (original): The method of claims 44 or 45, wherein the microanalytical device is fabricated by a method selected from the group consisting of silicon micromachining, microlithography, molding and etching.

Claim 56 (original): The method of claim 45, wherein the sol-gel is formed in situ on the microanalytical device.

Claim 57 (withdrawn): In a microanalytical device comprised of a substrate and at least one feature selected from microchannels, microcolumns, and combinations thereof, the improvement which comprises incorporating into said at least one feature particulates of sol-gel having a diameter of from about 10 μm to about 80 μm .

Claim 58 (original): In a microanalytical device comprised of a substrate and at least one feature selected from microchannels, microcolumns, and combinations thereof, the improvement which comprises incorporating into said at least one feature and/or onto a surface of the substrate a sol-gel having a biological molecule entrapped therein, wherein the sol-gel is in a form selected from the group consisting of a monolithic gel, a thin film, and a fiber.

Claim 59 (currently amended): The microanalytical device of claim ~~claims 57 or~~ 58, adapted for performing high throughput screening of samples.